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Lyme Disease Diagnosed by Alternative Methods: A Phenotype Similar to That of Chronic Fatigue Syndrome

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Background. A subset of patients reporting a diagnosis of Lyme disease can be described as having alternatively diagnosed chronic Lyme syndrome (ADCLS), in which diagnosis is based on laboratory results from a nonreference Lyme specialty laboratory using in-house criteria. Patients with ADCLS report symptoms similar to those reported by patients with chronic fatigue syndrome (CFS).

Methods. We performed a case-control study comparing patients with ADCLS and CFS to each other and to both healthy controls and controls with systemic lupus erythematosus (SLE). Subjects completed a history, physical exam, screening laboratory tests, 7 functional scales, reference serology for Lyme disease using Centers for Disease Control and Prevention criteria, reference serology for other tick-associated pathogens, and cytokine expression studies.

Results. The study enrolled 13 patients with ADCLS (12 of whom were diagnosed by 1 alternative US laboratory), 25 patients with CFS, 25 matched healthy controls, and 11 SLE controls. Baseline clinical data and functional scales indicate significant disability among ADCLS and CFS patients and many important differences between these groups and controls, but no significant differences between each other. No ADCLS patient was confirmed as having positive Lyme serology by reference laboratory testing, and there was no difference in distribution of positive serology for other tick-transmitted pathogens or cytokine expression across the groups.

Conclusions. In British Columbia, a setting with low Lyme disease incidence, ADCLS patients have a similar phenotype to that of CFS patients. Disagreement between alternative and reference laboratory Lyme testing results in this setting is most likely explained by false-positive results from the alternative laboratory.

Keywords. chronic fatigue syndrome; Lyme disease; case-control study; laboratory methods; clinical assessment.

Lyme disease, a tick-borne infection caused by *Borrelia burgdorferi* [1], results in >30 000 reported cases each year in the United States [2] and approximately 500 cases annually in Canada [3]—figures that likely underestimate the true incidence of the disease. Among patients reporting a diagnosis of Lyme disease, we observe 4 distinct groups (Table 1), largely differentiated by the method of diagnosis [4, 5]. One of these is the controversial category of alternatively diagnosed chronic Lyme syndrome (ADCLS), in which diagnoses are made on clinical grounds supported not by testing at a regional reference laboratory, but rather by Western blot (WB) testing performed at a nonreference Lyme specialty laboratory in the United States (Lab A). Such tests have been the subject of warnings with respect to their accuracy [6], offer no benefit in finding Lyme disease when it is present, and may produce false-positive results for >50% of people without Lyme disease who are tested [7, 8].

The province of British Columbia, like other regions in the Pacific Northwest, has a lower prevalence of *B. burgdorferi*–infected ticks and lower rates of Lyme disease than the Northeastern United States [9, 10]. In March 2013, British Columbia launched a clinic to

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Table 1. Four Distinct Subsets of the Lyme Disease Patient Population

Patient Category	Basis for Diagnosis
Undisputed Lyme disease	Diagnosed on appropriate clinical grounds in early disease or by reference laboratory testing in disseminated Lyme disease [4]
Posttreatment chronic Lyme syndrome	Diagnosed as above but failing to experience complete symptom resolution after standard antibiotic therapy [5]
Alternatively diagnosed chronic Lyme syndrome	Diagnosed on clinical grounds supported only by alternative tests, the validity of which is questioned by major reference laboratories and the Centers for Disease Control and Prevention [6]
Seronegative Lyme disease	Diagnosed on purely clinical grounds (a controversial category outside of early disease)

assist patients with suspected Lyme disease, along with patients diagnosed with chronic fatigue syndrome (CFS) and fibromyalgia. More than 350 000 Canadians report a diagnosis of CFS [11, 12], a debilitating syndrome of unknown etiology, characterized by profound fatigue exacerbated by physical or mental activity, impaired sleep, cognitive complaints, pain (myalgia, arthralgia, or headache), gastrointestinal symptoms, and/or tender lymph nodes [13]—all of which are symptoms similar to those reported by patients with ADCLS.

We leveraged the clinic opening to launch the Complex Chronic Disease Study, a multipart case-control study with the ultimate goals of producing detailed clinical comparisons of ADCLS and CFS and generating hypotheses about the diseases' etiologies. We recruited patients with ADCLS and CFS, as well as both healthy controls and controls with systemic lupus erythematosus (SLE), a chronic disease with accepted diagnostic criteria [14] in which patients frequently experience fatigue. Herein we report our initial findings arising from an extensive clinical comparison between the 74 recruited study participants.

METHODS

Recruitment

Institutional review board (IRB)–approved advertisements were posted in libraries, community and seniors' centers, and online through social media platforms and websites targeting patients with ADCLS and CFS. Some participants from patient groups learned of the study by word of mouth. We excluded participants if they were aged <19 years, unable to understand English, diagnosed with another medical condition explaining their symptoms, or on antibiotic therapy in the last month.

Diagnosis of ADCLS required all of the following criteria: (1) compatible symptoms according to alternative guidelines [15]; (2) residence in an area in which *B. burgdorferi* is endemic in ticks, including southern British Columbia, and/or a history of a rash compatible with erythema migrans; and (3) a diagnosis of Lyme disease by Lab A: either a positive serological test or a *Borrelia*-positive band at 31 or 34 kDa on a WB, and/or other diagnostic criteria used by the nonreference laboratory, such as a reduced CD57 cell count ($<0.060 \times 10^9$ cells/L). No patients belonging to the other 3 Lyme disease groups in Table 1 were identified during recruitment.

Patients with CFS had to meet the Canadian case definition [16], and we noted if they also met the Centers for Disease Control and Prevention (CDC) Fukuda definition [17]. The Canadian definition places greater emphasis on postexertional symptom exacerbation as a cardinal feature and defines a more specific subset of patients, and some of its attributes have been incorporated into the newly recommended US Institute of Medicine case definition [18].

Healthy controls were recruited as described above, and both healthy controls and SLE patients meeting American College of Rheumatology criteria [14] were matched by sex and 5-year age strata to CFS participants.

Subject Screening

We verified case definitions for each group by checklist and collected demographics, exposures, and full medical histories, and a complete physical examination was conducted by a physician (D. M. P., K. S., or Brian Ng). Heart rate and blood pressure were measured lying and after 60 seconds of standing, with postural hypotension defined as a drop in systolic blood pressure by ≥20 mm Hg or in diastolic blood pressure by ≥10 mm Hg [19]; postural tachycardia was defined as an increase from lying to standing heart rate of ≥30 beats per minute [20]. Laboratory screening tests included complete blood count, calcium, phosphorous, magnesium, glucose, electrolytes, thyroid-stimulating hormone (TSH), ferritin, urea, creatinine, creatinine kinase, uric acid, liver function, anti-DNA antibodies, antinuclear antibodies, extractable nuclear antigens, C-reactive protein, rheumatoid factor, lactate, acyl carnitine, protein, albumin, protein electrophoresis, rapid plasma reagin, human immunodeficiency virus, and hepatitis B and C serology. Patients from any group were excluded from the study if any screening tests identified an alternative diagnosis to explain symptoms; these are listed with standard research case definitions for CFS [13, 17].

Study Protocol

The protocol was approved by the University of British Columbia's IRB (H11-01998).

Subjects completed the Karnofsky performance status scale [21], the 36-Item Short-Form Health Survey (SF-36; Physical and Mental) [22], the Pittsburgh Sleep Quality Index [23], the Center for Epidemiological Studies Depression Scale [24], the

Fatigue Severity Scale [25], and a Functional Capacity Scale widely used by CFS clinicians [26].

Study laboratory work included complement C3 and C4; CH50 alternative and CH50 classical assays; and CD3, CD4, CD8, and CD57 cell fractions and counts. For the purposes of this study, reference Lyme serology refers to 2-tier testing using CDC criteria [27, 28]. Screening was done using the VIDAS Lyme immunoglobulin G (IgG) and immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA; bioMérieux SA). IgM and IgG WB (MarDx Diagnostic Inc) were performed on all positive and equivocal samples. We also conducted additional testing using a WB with antibodies reactive to European *Borrelia* strains *(B. afzelii*, *B. garinii*) and C6 peptide ELISA (Immunetics) [19].

Serological tests for other tick-associated pathogens included immunofluorescence assays for *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, *Rickettsia rickettsii*, *Coxiella burnetii*, *Bartonella henselae*, *Bartonella quintana*, and *Babesia microti* serology, and a microagglutination assay for *Francisella tularensis* serology.

For lipopolysaccharide (LPS)–stimulated cytokine quantification, 1 mL of heparinized whole blood was inoculated at 200 µL per well in round-bottom 96-well plates and stimulated with 30 ng/mL of *Escherichia coli* LPS for 21–23 hours. Plasma from all of the wells was combined and frozen at −80°C until tested. Unstimulated cytokines were measured in plasma separated from freshly drawn heparinized blood and frozen at −80°C until testing. Cytokine concentrations were measured using custom plates (Meso Scale Diagnostics) according to the manufacturer's recommended protocol, and duplicate samples were averaged.

Sample Size

Sample size was structured to suit the hypothesis-generating purposes of the transcriptomic and metagenomic analyses that form another arm of the Complex Chronic Disease Study (data not shown) [29, 30] (C. Chiu, personal communication). In the present analysis, our study had power of 0.83 to detect a 10-point difference in Karnofsky or SF-36 scores between the CFS and ADCLS groups.

Data Analysis

Univariate analyses were performed using Fisher exact test for categorical and Wilcoxon rank-sum tests for continuous variables. In lieu of the Bonferroni correction—conservative given the correlated nature of many of the outcomes—we put greater interpretative weight on the magnitude and consistency of differences.

RESULTS

We screened 161 people and excluded 87 (28 with another diagnosis, 21 who did not meet the case definitions, 13 who declined to participate, 7 on antibiotic therapy, 3 lost to follow-up, and 15 eligible patients who could not be matched to a control). The final cohort comprised 13 ADCLS cases, 25 CFS cases, 25 healthy controls, and 11 SLE controls. Twelve patients with ADCLS reported a positive serological result from Lab A; the remaining patient reported a positive band at 31 kDa on a WB from Lab A.

Demographics, Symptoms, and Putative Triggers

We first examined cases and controls for differences in demographics, symptoms, and putative triggers associated with symptom onset (Table 2). ADCLS subjects were a median of 8 years younger than CFS and control patients ($P = .02$). Educational and income levels were similar across the groups, with a nonsignificant trend to lower median income in ADCLS. CFS patients were more likely to be white than controls (92% vs 80%, respectively; $P = .04$), with a similar nonsignificant trend in that direction for ADCLS.

ADCLS and CFS patients were significantly more likely to report core symptoms (fatigue, body pain, nonrefreshing sleep) than healthy controls; however, there were no significant differences between the ADCLS and CFS groups with respect to symptoms. Additionally, all of the CFS patients and 85% of ADCLS patients met the CDC Fukuda case definition for CFS [17]. SLE controls were clearly distinguishable from healthy controls, but reported fewer fatigue- and dysfunction-related symptoms relative to case patients.

Only 2 ADCLS patients indicated a history of tick bite or skin rash at symptom onset; however, 54% of ADCLS, 44% of CFS, and 27% of SLE patients reported viral illness as a trigger. There were no significant differences between the CFS and ADCLS groups in reporting any triggering event.

Functional Scales

On all 7 functional scales, ADCLS and CFS patients scored poorly compared with healthy controls (Figure 1). SLE patients scored significantly worse than healthy controls on 4 scales, but with a distribution of scores closer to healthy controls. The only scale for which we observed a significant difference between ADCLS and CFS patients was the Fatigue Severity Scale, on which CFS patients reported higher fatigue severity than ADCLS patients ($P = .02$).

History and Physical Examination

ADCLS, CFS, and SLE groups had significantly more positive responses to history questions than did controls (Supplementary Data). Subjects with ADCLS and CFS more frequently reported depression, anxiety, and other emotional issues than healthy or SLE subjects, but not to a statistically significant degree. ADCLS and CFS patients also reported significantly more cognitive complaints vs healthy controls, as well as significantly more physical complaints, particularly respiratory, gastrointestinal, musculoskeletal, and genitourinary issues. Subjects with SLE

Table 2. Demographic and Clinical Characteristics of the Study Cohort (N = 74)

Group	Healthy (n = 25)	SLE (n = 11)	CFS (n = 25)	ADCLS (n = 13)	*P* Value			
					SLE vs Healthy	CFS vs Healthy	ADCLS vs Healthy	CFS vs ADCLS
Male sex	4 (16)	0 (0)	4 (16)	3 (23)	.3	1.0	.7	.7
Age, y, median (IQR)	53 (30–69)	51 (29–75)	54 (34–67)	45 (18–71)	.5	.9	**.02**	**.02**
Highest level of education					.4	.7	.9	1.0
High school	4 (16)	2 (18)	6 (24)	3 (23)				
Undergraduate	16 (64)	9 (82)	13 (52)	7 (54)				
Postgraduate	5 (20)	0 (0)	6 (24)	3 (23)				
Current annual income, median (IQR)	$35 000 ($15 000– $55 000)	$45 000 ($12 500–$55 000)	$35 000 ($17 500–$65 000)	$22 500 ($2,500–$45 000)	.7	.8	.4	.3
Ethnicity					.08	**.04**	.4	.4
Aboriginal	0 (0)	0 (0)	2 (8)	0 (0)				
White	20 (80)	5 (45)	23 (92)	13 (100)				
Chinese	3 (12)	3 (27)	0 (0)	0 (0)				
Other	2 (8)	3 (27)	0 (0)	0 (0)				
Symptom onset sudden	NA	4 (36)	13 (52)	3 (23)	NA	NA	NA	.2
Core symptoms								
Fatigue	4 (16)	9 (82)	25 (100)	12 (92)	**<.0001**	**<.0001**	**<.0001**	.3
Postexertional fatigue	2 (8)	5 (45)	25 (100)	11 (85)	**.02**	**<.0001**	**<.0001**	.1
Nonrefreshing sleep or sleep disturbance	8 (32)	8 (73)	25 (100)	12 (92)	**.03**	**<.0001**	**.001**	.3
Pain or headache	15 (60)	10 (91)	25 (100)	13 (100)	.1	**.001**	**.008**	1.0
Neurological/cognitive dysfunction	1 (4)	5 (45)	25 (100)	11 (85)	.006	**<.0001**	**<.0001**	.1
Swollen joints	1 (4)	5 (45)	8 (32)	7 (54)	**.006**	**.02**	**.001**	.3
Painful joints	7 (28)	8 (73)	17 (68)	12 (92)	**.03**	**.01**	**<.0001**	.1
Meeting Fukuda CFS definition	0 (0)	0 (0)	25 (100)	11 (85)	1.0	**<.0001**	**<.0001**	.1
Putative triggers associated with symptom onset								
Viral illness	NA	3 (27)	11 (44)	7 (54)	NA	NA	NA	.7
Bacterial infection	NA	1 (9)	4 (16)	3 (23)	NA	NA	NA	.7
Tick bite	NA	0 (0)	4 (16)	2 (15)	NA	NA	NA	1.0
Skin rash	NA	5 (45)	2 (8)	2 (15)	NA	NA	NA	.6

Values are presented as No. (%) for categorical variables and median (IQR) for continuous variables. *P* values were calculated with Fisher exact test for categorical variables or Wilcoxon rank-sum test for continuous variables.

Bold values denote statistically significant at $P < .05$.

Abbreviations: ADCLS, alternatively diagnosed chronic Lyme syndrome; CFS, chronic fatigue syndrome; IQR, interquartile range; NA, not applicable; SLE, systemic lupus erythematosus.

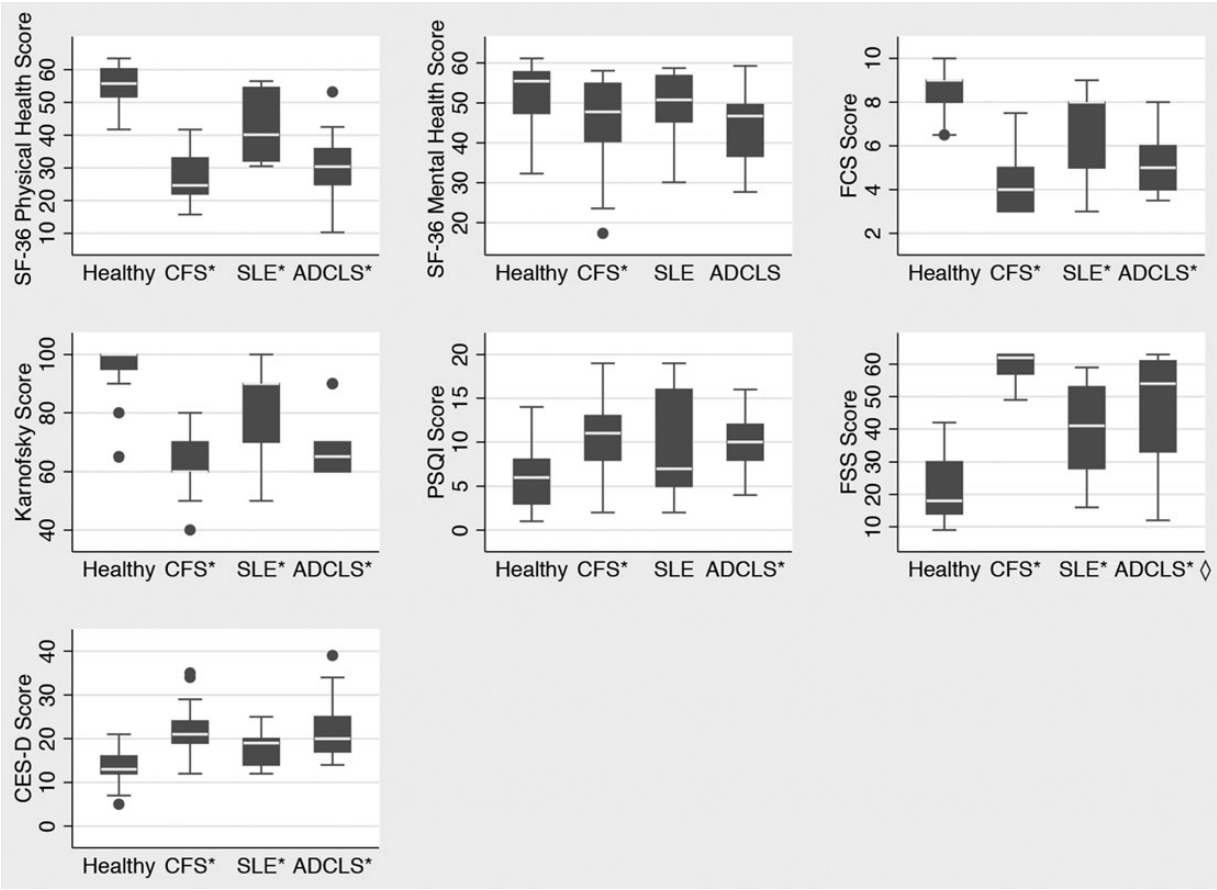

Figure 1. Functional scales by patient group. *Significant difference from healthy control. ◊Significant difference between chronic fatigue syndrome (CFS) and alternatively diagnosed chronic Lyme syndrome (ADCLS) ($P < .05$). Center line in box represents the median and box edges represent the interquartile range. Outer lines represent upper and lower adjacent values, and dots represent outside values. Abbreviations: CES-D, Center for Epidemiological Studies Depression Scale; FCS, Functional Capacity Scale; FSS, Fatigue Severity Scale; PSQI, Pittsburgh Sleep Quality Index; SF-36, Medical Outcomes Study 36-Item Short-Form Health Survey; SLE, systemic lupus erythematosus.

reported fewer differences vs healthy controls, but noted significantly more cognitive complaints, sore throat, and swollen or painful joints. There were no significant differences between ADCLS and CFS patients in the reporting of any symptom, except for more frequent sore throats in CFS.

On physical examination (Supplementary Data), CFS patients had a higher body mass index (BMI) and waist circumference than healthy controls ($P < .05$). There was no significant evidence of increased orthostatic intolerance in case patients, as elsewhere reported in CFS [31], nor were there differences in Romberg or rapid alternating movement. Otherwise, motor, sensory, and reflex examinations did not differ between these 2 groups. ADCLS and CFS patients had significantly more fibromyalgia tender points [32] and tender joints than healthy controls.

Laboratory Findings

No study subjects, including patients with ADCLS, tested positive for Lyme disease using reference serological criteria, and there was no significant difference between the groups in serology for other tick-borne infections. There were no significant differences in hematological parameters between ADCLS and CFS patients and healthy controls (Table 3), although the SLE group had lower absolute CD4 ($P = .004$) and $CD3^{-}CD57^{+}$ natural killer ($P = .03$) cell subsets and more detectable antinuclear antibody ($P = .01$). Screening tests for other disorders did not differ between any case or control groups. Two healthy controls and 1 CFS patient had abnormal TSH, but in each case the TSH was marginally low with no symptoms of uncorrected thyroid disease.

Cytokine Expression

As expected, subjects with SLE exhibited higher expression of several cytokines in the unstimulated samples (Supplementary Data). By contrast, there was little to differentiate ADCLS and CFS patients from healthy controls or from each other, except that unstimulated interleukin 6 was marginally higher (median,

Table 3. Laboratory Results From the Study Cohort (N = 74)

Group	Healthy (n = 25)	SLE (n = 11)	CFS (n = 25)	ADCLS (n = 13)	*P* Value			
					SLE vs Healthy	CFS vs Healthy	ADCLS vs Healthy	CFS vs ADCLS
Hemoglobin level	134 (128–144)	133 (125–136)	137 (133–142)	133 (129–137)	.5	.1	.9	.2
WBC count	5.5 (4.7–7.1)	5.1 (4.7–5.7)	6.0 (5.5–6.5)	5.4 (5.0–6.8)	.3	.4	1.0	.5
CD3	0.74 (0.69–0.82)	0.73 (0.67–0.80)	0.73 (0.70–0.79)	0.75 (0.70–0.82)	.6	.5	.6	.3
CD3 absolute	1.27 (1.00–1.50)	1.00 (0.87–1.16)	1.33 (0.95–1.69)	1.35 (1.22–1.69)	.05	.9	.3	.4
CD4	0.52 (0.46–0.57)	0.47 (0.36–0.55)	0.52 (0.48–0.61)	0.52 (0.49–0.58)	.1	.8	1.0	.8
CD4 absolute	0.92 (0.66–1.01)	0.59 (0.44–0.73)	0.96 (0.66–1.22)	0.94 (0.74–1.26)	**.004**	.6	.3	.6
CD8	0.22 (0.18–0.28)	0.30 (0.20–0.36)	0.20 (0.15–0.23)	0.25 (0.19–0.29)	.06	.2	.6	.08
CD8 absolute	0.39 (0.28–0.44)	0.40 (0.27–0.54)	0.33 (0.26–0.44)	0.49 (0.29–0.60)	.7	.4	.3	.08
CD57	5.5 (2.2–8.6)	2.3 (1.7–6.8)	5.0 (2.9–7.3)	5.1 (2.9–7.9)	.1	.4	.8	.8
CD57 absolute	0.10 (0.05–0.14)	0.04 (0.02–0.08)	0.09 (0.06–0.10)	0.10 (0.07–0.16)	**.03**	1.0	.6	.5
Acyl carnitine	12.3 (9.25–13.8)	10.8 (8.5–16.9)	12.7 (9.1–15.1)	11.0 (9.4–13.0)	.9	.5	.5	.7
CRP (censored at 0.2)	0.6 (0.3–1.4)	1.2 (0.3–3.0)	1.2 (0.6–2.6)	0.4 (0.2–1.2)	.5	.1	.6	.1
TSH (abnormal)	2 (8)	0 (0)	1 (4)	0 (0)	1.0	1.0	.5	1.0
Glucose	5.1 (4.6–5.5)	4.5 (4.1–4.9)	5.2 (4.7–5.6)	5.2 (4.8–5.4)	.07	.7	.9	.8
RF (abnormal)	0 (0)	1 (9)	1 (4)	1 (8)	.3	1.0	.3	1.0
ANA (positive)	6 (24)	8 (73)	3 (12)	3 (23)	**.01**	.5	1.0	.4
Reference Lyme serology (reactive)	0 (0)	0 (0)	0 (0)	0 (0)	. . .	. . .	. . .	. . .
C6 peptide IgG reactive	0 (0)	2 (18)	0 (0)	1 (8)	.09	. . .	.3	.3
C6 Peptide IgM reactive	0 (0)	2 (18)	0 (0)	1 (8)	.09	. . .	.3	.3
Anaplasma phagocytophilum	2 (8)	1 (9)	1 (4)	2 (15)	1.0	1.0	.6	.3
Ehrlichia chaffeensis	0 (0)	1 (9)	0 (0)	1 (8)	.3	. . .	.3	.3
Rickettsia rickettsii	0 (0)	1 (9)	0 (0)	0 (0)	.3	. . .	. . .	. . .
Coxiella burnetii Q fever phase I IgG	0 (0)	0 (0)	1 (4)	0 (0)	. . .	1.0	. . .	1.0
Coxiella burnetti Q fever phase II IgG	0 (0)	0 (0)	1 (4)	0 (0)	. . .	1.0	. . .	1.0
Bartonella henselae[a]	0 (0)	0 (0)	0 (0)	0 (0)	. . .	. . .	. . .	. . .
Babesia microti	0 (0)	0 (0)	0 (0)	0 (0)	. . .	. . .	. . .	. . .
Francisella tularensis	0 (0)	0 (0)	0 (0)	0 (0)	. . .	. . .	. . .	. . .

Values are No. (%) for categorical variables and median (IQR) for continuous variables. *P* values were calculated with Fisher exact test for categorical variables or Wilcoxon rank-sum test for continuous variables.

Bold values denote statistically significant at $P < .05$.

Abbreviations: ADCLS, alternatively diagnosed chronic Lyme syndrome; ANA, antinuclear antibody; CFS, chronic fatigue syndrome; CRP, C-reactive protein; IgG, immunoglobulin G; IgM, immunoglobulin M; RF, rheumatoid factor; SLE, systemic lupus erythematosus; TSH, thyroid-stimulating hormone; WBC, white blood cell.

[a] *Bartonella henselae* serology not done for 1 SLE patient.

0.51 vs 0.41 pg/mL) in CFS vs healthy controls ($P = .02$), and monocyte chemoattractant protein-1 was marginally lower (median, 115 vs 143 pg/mL) in ADCLS patients than in CFS patients ($P = .04$).

DISCUSSION

Consistent with other reports on CFS [12, 33, 34], our subjects with both ADCLS and CFS report an inability to meet the demands of a full-time job, sleep disturbance, and profound fatigue and are clearly distinct from both healthy and SLE controls with respect to most of the variables examined. Strikingly, ADCLS and CFS patients appear indistinguishable based on their medical histories, physical examination, functional scales, and a range of laboratory tests. Cytokine differences between groups were not significant given the number of comparisons. Taken collectively, these findings suggest that the primary difference between ADCLS and CFS groups lies in differing diagnostic approaches. Twelve of 13 ADCLS patients had a diagnosis supported with positive serology at Lab A: 4 on IgM WB alone, 4 on IgG WB alone, and 4 on both assays. Both the IgG and IgM WBs from Lab A reported bands that the reference laboratory examined but did not find to be positive; furthermore, Lab A's IgM WBs reported additional bands not used in reference testing because of concerns about their specificity [6].

Tests with imperfect specificity yield an increasing proportion of false-positive results when applied to populations with a low prevalence of the target disease. Independent evaluation has found that specificity at alternative laboratories can be <50% [7]. In British Columbia, Lyme disease prevalence in the tested population is well below 1%, meaning that false-positive diagnoses from an alternative laboratory can exceed true positives by a ratio of at least 50 to 1. Our observation of 12 patients with an alternative Lyme diagnosis unconfirmed by reference testing would be a probable event, estimated as P (false positive)^{12} or $0.98^{12} = 78\%$. If we accept the alternative position that reference testing is only 40% sensitive, the probability that 12 sequential patients would fail to register on all reference testing can be given as (1 – sensitivity)^{12} or $0.6^{12} = 0.2\%$. Thus, it is far more likely that false-positive tests from Lab A explain our observations than false-negative reference tests. Our findings are consistent with previous studies reporting overdiagnosis of Lyme disease using alternative methods in our region [35]. Some practitioners employ CD57 cell count as an additional criterion to support diagnosis of ADCLS. Our finding of no significant difference in CD57 cell counts between ADCLS and CFS patients and healthy controls is consistent with another study [36] that could not replicate the findings on which such practice is based [37].

This study has certain limitations. A small sample size provides low power to detect small differences between groups. Case-control studies may suffer from recall bias as a function of strong personal identification with a diagnosis and its associated risk factors and symptoms. Selection bias is a risk, but was minimized by using identical exclusion criteria across all study groups. Inclusion of 4 groups in our study meant that it was feasible to match by age for only 3 of them. A lower median age in the ADCLS group is a limitation and may explain lower BMI in that group compared to CFS. For ADCLS, case definitions have often been constructed with the goal of catching any possible case. This risks misclassification, making it harder to identify associations that lead to a better understanding of etiology. We employed a definition that best identifies the patient group for which there has been the greatest controversy in our region and have demonstrated that although their diagnosis with Lyme disease is questionable, most ADCLS subjects can meet a research definition for inclusion in studies of CFS. We also made considerable effort to recruit posttreatment chronic Lyme syndrome (PTCLS) subjects, without success. It is possible that patients with PTCLS avoid mainstream clinics, but our experience more likely reflects low prevalence of PTCLS and mitigation of symptoms in most people treated for undisputed Lyme disease. Our findings may not be generalizable to higher-prevalence areas for Lyme disease. However, tick ecology and prevalence are similar between British Columbia and the states of Washington and Oregon, making our conclusions relevant for these geographically similar regions.

We conclude that false-positive serological results from an alternative Lyme specialty laboratory represent the most likely reason for different labels for what is clearly a debilitating illness. Individuals diagnosed with ADCLS deserve comprehensive workup and care. Many will meet case definitions for CFS and should be included in studies employing metagenomics, transcriptomics, and other approaches in the search for a more plausible etiology [38].

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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